アセチルコリンの冠注で誘発されるブタの主および小冠動脈攣縮モデルの開発とこのモデル動物を用いたニプラジロール, イソソルビド・ジニトレート, ブナゾシンの冠攣縮予防効果に関する研究(Dissertation_全文)

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アセチルコリンの冠注で誘発されるブタの主および小冠動脈攣縮モデルの開発とこのモデル動物を用いたニプラジロール、イソソルビド・ジニトレート、ブナゾシンの冠攣縮予防効果に関する研究

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Response of large and small coronary arteries of pigs to intracoronary injection of acetylcholine: angiographic and histologic analysis

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Kawamura A, Fujiwara H, Onodera T, Wu D-J, Matsuda M, Ishida M, Takemura G, Fujiwara Y, Kawai C. Response of large and small coronary arteries of pigs to intracoronary injection of acetylcholine: angiographic and histologic analysis. Int J Cardiol 1989;25:289–302.

With coronary arteriography we examined the effect of acetylcholine (ACh) on large and small coronary arteries. ACh (12.5 to 200 μg) was injected into the right coronary arteries of 10 pigs during left ventricular pacing. The percentage of narrowing of the epicardial major coronary artery was used as an indicator of the constriction of the large coronary arteries, and the time required for the contrast medium to reach the posterior descending coronary artery from the ostium of the right coronary artery (blood-flow delay) was used as an indicator of the constriction of the small coronary arteries. A small dose of ACh (12.5 to 100 μg) induced mild narrowing (14 to 41%) of the epicardial major coronary artery and a marked blood-flow delay of over 7.0 sec (control: ≤ 1.8 sec) in all 10 pigs. A large dose of ACh (100 to 200 μg) caused over 75% narrowing of the epicardial major coronary artery and a marked blood-flow delay in 4 of the 10 pigs. When the marked blood-flow delay appeared, the perfused right ventricular myocardium became macroscopically anemic (ischemic). The constriction of large and small coronary arteries was not prevented by diphenhydramine (H1 blocker: 100 mg i.v.), but was prevented by pretreatment with atropine (1.0 mg i.v.). The intracoronary injection of histamine (1.5 mg) in 5 pigs constricted the epicardial major coronary artery over 75% in 2 pigs, 50 to 75% in 1 pig, and 25 to 50% in 2 pigs, but there was no evidence of blood-flow delay. Neither methoxamine nor norepinephrine caused any significant coronary artery narrowing. The histology of the large and small coronary arteries was examined quantitatively with an image analyzer. The coronary artery showed no intimal thickening, and the endothelium was intact on light microscopic examination. The % area of the smooth muscle layer (media) to the calculated total vascular area, and the ratio of the calculated medial thickness to the calculated inner radius (h/Ri) were 64 ± 7% (mean ± SD) and 0.69 ± 0.16, respectively, in the small coronary arteries less than 100 μm in external diameter, 47 ± 9% and 0.39 ± 0.12 in the small coronary arteries 100 to 2000 μm in external diameter, and 34 ± 4% and 0.24 ± 0.03 in the large right coronary arteries over 2000 μm in external diameter; the % area of the media and the h/Ri showed a negative correlation with the size of the coronary arteries. These findings indicate that ACh constricts

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Protective effects of nipradilol, isosorbide dinitrate, and bunazosin on coronary artery constriction induced by intracoronary injection of acetylcholine in pigs

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Abstract

Study objective – **Protective effects of nipradilol, a newly synthesised vasodilating β adrenoceptor antagonist, isosorbide dinitrate, and bunazosin on coronary artery constriction induced by intracoronary injection of acetylcholine were determined by coronary arteriography and compared in vivo in pigs.**

Design – **Acetylcholine (12.5, 25, 50, 100 and 200 μg) was given into the right coronary artery under left ventricular pacing to maintain constant systemic haemodynamics. Percentage narrowing of the major epicardial coronary artery was used as an indicator of constriction of the large coronary arteries, and the time required for the contrast medium to reach the posterior descending coronary artery from the ostium of the right coronary artery (blood flow delay) was used as an indicator of constriction of the small coronary arteries.**

Subjects – **15 farm pigs weighing 80 to 90 kg were used.**

Measurements and main results – **A marked blood flow delay of over 7.0 s (control: ≤1.8 s) with less than 34% narrowing of the epicardial major coronary artery was observed in 13 of 15 pigs with 12.5-50 μg of acetylcholine, and in the other two pigs with 100 μg of acetylcholine. When marked blood flow delay**

occurred, the perfused right ventricular myocardium became macroscopically anaemic (ischaemic). Over 75% narrowing of the major epicardial coronary artery was induced in six of the 15 pigs, and over 50% narrowing in 12, with marked blood flow delay with 100 to 200 μg of acetylcholine. However, after intracoronary infusion of 10 μg of nipradilol, acetylcholine induced narrowing in the epicardial major coronary artery was significantly reduced from 44-79% in control to 19-37% despite 200 μg of acetylcholine, though the time delay in coronary blood flow did not change significantly. By pretreatment with intracardiac isosorbide dinitrate (2.5 mg), the percent narrowing of the large coronary artery and the time delay in coronary blood flow were significantly reduced (narrowing from 32-84% to 10-27%; time delay from 7.6-41.6 s to 2.7-22.7 s). Pretreatment with intracardiac bunazosin, an α 1 adrenoceptor antagonist, (100 μg) showed no protective effect on narrowing of the epicardial major coronary artery or blood flow delay.

Conclusions—Isosorbide dinitrate prevents coronary artery constriction induced by acetylcholine in swine. Nipradilol prevents large, but not small, coronary artery constriction, probably through a direct nitrate like vasodilating action.

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Key words: coronary artery spasm; acetylcholine; nipradilol; isosorbide dinitrate (ISDN); bunazosin; pig

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Galley 1

The effectiveness of β adrenoceptor antagonists on effort angina and hypertension has been well established.[1-3] However, treatment with β adrenoceptor antagonists is not always beneficial, because they induce coronary artery spasm[4 5] and some cases of effort angina and/or hypertension are accompanied by vasospastic angina. Nipradilol, 3,4-dihydro-8(2-hydroxy-3-isopropylamino) propoxy-3-nitroxy-2H-1-benzopyran (fig 1), has been newly synthesised at the Tokyo Research Laboratories of Kowa Co, Japan. Nipradilol possesses non-slective β adrenoceptor blocking action, a weak β adrenoceptor blocking action, and nitroester group activity.[6] Nipradilol dilates coronary arteries in vitro.[7] However, its preventive effects on the constriction of coronary arteries have not been examined in vivo, since no good animal model of large and small coronary artery constriction has been found. The response of human large coronary arteries to acetylcholine is variable, ranging from dilatation to constriction in the normal large coronary artery.[8 9] However, acetylcholine generally constricts an abnormal large coronary artery[9 10] and induces spasm in most patients with variant angina.[11] In vitro, acetylcholine constricts porcine large coronary arteries,[12 13] and we recently reported an angiographic study showing that it constricts porcine large and small coronary arteries in vivo.[14] In the present study, using the same pig model, we examined angiographically whether nipradilol prevented the constriction of the large and small coronary arteries induced by acetylcholine. The effects of isosorbide dinitrate and bunazosin, an α1 antagonist,[15] were also examined for comparison.

Methods

ANIMAL PREPARATION

Fifteen farm pigs, weighing 80 to 90 kg were anaesthetised with intramuscular ketamine hydrochloride (12.5 mg·kg^{-1}), followed by intravenous sodium pentobarbitone (20 mg·kg^{-1}), and ventilated with a volume limited ventilator. To exclude coronary artery spasm caused by hyperventilation,[16] the ventilatory rate and inspired oxygen concentration were controlled to maintain arterial pH between 7.35 and 7.45, partial arterial oxygen pressure between 10.6 and 16.0 kPa (80-120 mm Hg), and partial arterial carbon dioxide pressure between 4.7 and 6.0 kPa (35-45 mm Hg). Limb leads of the electrocardiogram and arterial blood pressure were monitored continuously during the experiment.

EXPERIMENTAL PROTOCOL

The pigs were divided into nipradilol, isosorbide dinitrate, and bunazosin groups. Each group was composed of five pigs. The heart was exposed by median sternotomy and pacing wires were implanted in the left ventricular free wall. After administration of 20 000 units heparin intravenously, selective coronary arteriography was performed by injecting manually about 10 ml of warmed (36-37°C) contrast medium (Iopamiron® 370, Nihon Schering KK) through an 8F Sones catheter. Acetylcholine dissolved in 5 ml of

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warmed 0.9% saline solution was injected at increasing doses of 12.5, 25, 50, 100 and 200 μg into the right coronary artery every 10 min. Right coronary arteriography was performed within 3 s after each acetylcholine injection. When the degree of narrowing of the major epicardial right coronary artery was over 75%, the administration of acetylcholine was stopped. Fifteen minutes later, when the effects of myocardial ischaemia induced by acetylcholine infusion, such as anaemic colour and dyskinetic movement in the right ventricular wall, had disappeared completely, the drugs were then given into the right coronary artery. In the nipradilol group, 10 μg of nipradilol (Kowa Co, Tokyo), and in the bunazosin group, 100 μg of bunazosin (Eisai Co, Tokyo), dissolved in 5 ml of warmed 0.9% saline solution, were given into the right coronary artery. In the isosorbide dinitrate group, 2.5 mg of isosorbide dinitrate (Eisai Co, Tokyo) was injected into the right coronary artery. About 30 s later the same dose of acetylcholine was injected again into the right coronary artery, and right coronary arteriography was performed. To avoid brady-arrhythmias and hypotension during intracoronary injection of acetylcholine,[9] coronary arteriography was performed while the heart rate was kept physiologically constant by left ventricular pacing.

ASSESSMENT OF LARGE AND SMALL CORONARY ARTERY CONSTRICTION BY CORONARY ARTERIOGRAPHY

Coronary arteriograms were reviewed on a video TV screen by two independent, blinded observers. The luminal diameter of the epicardial major coronary artery was measured using an image analyser (Avio excel, Model TVIP-4100, Nippon Avionics Co) with automated edge detection at proximal, middle, and distal sites of the right epicardial major coronary artery in matched end diastolic frames. The narrowing of the epicardial major coronary artery was calculated as [(diameter before injection of ACh minus diameter after injection of ACh) ÷ diameter before injection of ACh] × 100. The maximum value among the above three sites was used as an indicator of large coronary artery constriction. The time required for the contrast medium to reach the posterior descending coronary artery from the ostium of the right coronary artery was measured and classified into three grades: marked delay: >7.0 s; slight delay: 1.8-7.0 s; and normal ≤1.8 s. Marked delay was defined as the time delay when myocardial ischaemia, such as anaemic colour change and dyskinetic wall motion in the right ventricle, appeared. Blood flow delay was used as an index of small coronary artery constriction, when the degree of narrowing of the major coronary artery was under 90% (see the section of animal model in Discussion).[14] To determine interobserver variability, the readings were performed blind by the observers conducting this study, using the video at different times. The variability between the two observers was ±5% for the narrowing of the epicardial major coronary artery, and ±0.3 s for the time of blood flow delay.

STATISTICAL ANALYSIS

Haemodynamic data for control and after administration of acetylcholine with or without each agent were compared by one way analysis of variance with multiple comparisons using a Bonferonni correction in each group ($p<0.05$; significant). The data on the coronary artery narrowing and on the time of blood flow delay in each group were analysed by the paired t test ($p<0.05$ significant). Data were expressed as mean values (SD).

Results

HAEMODYNAMIC CHANGES

Aortic systolic and diastolic pressures and heart rates did not change significantly after the injection of acetylcholine with or without nipradilol, isosorbide dinitrate, or bunazosin (table I).

EFFECTS OF NIPRADILOL, ISOSORBIDE DINITRATE AND BUNAZOSIN ON LARGE AND SMALL CORONARY ARTERY CONSTRICTION INDUCED BY ACETYLCHOLINE

In all pigs, a delay in coronary blood flow and a narrowing of the epicardial major coronary artery occurred dose dependently after injections of acetylcholine ranging from 12.5 to 200 μg (figs 2 A,B; tables II-IV), and they subsided spontaneously within 5 min. However, a marked delay in coronary blood flow was noted in each pig when the narrowing of the epicardial major coronary artery was slight (34% or less).

Macroscopically, the myocardium (right ventricular wall) supplied by the right coronary artery became anaemic (ischaemic) and was clearly demarkated from the area supplied by the left coronary artery, when a marked delay (the time delay of blood flow >7.0 s) in right coronary arterial flow was noted (fig 3). After the administration of 10 μg of nipradilol into the right coronary artery, narrowing of the epicardial major right coronary artery induced by 200 μg acetylcholine reduced from 44-79%, before the administration of nipradilol, to 19-37% ($p<0.01$, fig 1 C). The ratio of percent narrowing following pretreatment with nipradilol to that of control without nipradilol at a maximum dose of 200 μg acetylcholine percent constriction of the large coronary artery with nipradilol) was 49(8)% (38-56%) ($p<0.01$). However, there was no significant difference in the time delay in coronary blood flow with or without nipradilol; the ratio of the time delay in coronary blood flow following pretreatment with nipradilol to that of control administration of acetylcholine without nipradilol (percent constriction of the small coronary artery with nipradilol) was 95(9)% (86-108%).

After the intracoronary administration of 2.5 mg of isosorbide dinitrate, the narrowing in the major epicardial coronary artery at the same dose of acetylcholine (100 μg in one pig and 200 μg in the other four pigs) changed from 32-84% in the control to 10-27% ($p<0.01$); the percent constriction of the large coronary artery with isosorbide dinitrate was 28(4)% (21-32%) ($p<0.01$). With isosorbide dinitrate pretreatment, the time delay in coronary blood flow was significantly reduced to 53(21)% (26-76%) of the control value when acetylcholine was given without isosorbide dinitrate; the percent constriction of the small coronary artery with isosorbide dinitrate was 53(21)% ($p<0.05$).

In each pig of the bunazosin group, despite pretreatment with intracardiac bunazosin (100 μg), the same dose of intracardiac acetylcholine (200 μg) induced a similar narrowing of the major epicardial coronary artery and similar blood flow delay as the control without bunazosin pretreatment (fig 4).

Discussion

ANIMAL MODEL

Injection of acetylcholine into the left coronary artery almost always induced ventricular fibrillation and death in pigs. Therefore, acetylcholine was selectively given into the right coronary arteries. To avoid bradyarrhythmias and hypotension after the intracoronary injection, a pacemaker was implanted in the left ventricular wall. As a result, the haemodynamics were not altered significantly by the administration of acetylcholine, with or without nipradilol, isosorbide dinitrate, or bunazosin. We do not know whether the present data from the right coronary artery can apply directly to the left coronary artery.

In the present study, we used angiographic narrowing of the major epicardial coronary artery as an indicator of constriction of large coronary arteries, and delay in the coronary blood flow without significant (more than 90%) narrowing of the major epicardial coronary artery as an indicator of constriction of small coronary arteries. The time delay as an index of small coronary artery constriction is influenced by large coronary artery responses, when severe narrowing occurs in a large coronary artery. In our previous study, intracoronary injection of histamine did not induce any time delay in coronary blood flow despite 75-90% narrowing of the epicardial major coronary artery.[14] In the present study, small doses of acetylcholine induced a marked delay in coronary blood flow despite mild narrowing (≤34%) in the epicardial major coronary artery, and even at large doses of acetylcholine (100 or 200 μg), a marked delay in coronary blood flow was seen with less than 84% epicardial major coronary artery narrowing. The time delay in coronary blood flow in the present study therefore reflects small coronary artery constriction. Although the reproducibility of the appearance times is good, the methods in the present study are not quantitative with regard to the relationship between coronary arterial pressure and blood flow. However, swine coronary artery was irritable to mechanical stimuli and induced spasm and ventricular fibrillation were easily induced. Therefore, we did not use any devices to measure coronary blood flow.

The contrast medium (Iopamiron®370, Nihon Schering KK) used in the present study is non-ionic, and has haemodynamic effects such as vasodilatation, although the degree of vasodilatation is less than that caused by ionic contrast media.[17] However, it was impossible to observe this phenomenon with the method of coronary arteriography used. Indicators of large and small coronary artery constriction were therefore analysed together with control arteriograms, which were performed using infusion of contrast medium alone, and with arteriograms using contrast medium with or without each agent after injection of acetylcholine.

COMPARISON OF [illegible]

COMPARISON OF EFFECTS OF NIPRADILOL, ISOSORBIDE DINITRATE AND BUNAZOSIN ON CONSTRICTION OF LARGE AND SMALL CORONARY ARTERIES

In previous human and animal studies, the doses of vasoactive drugs which were given into the right coronary artery in the present study were sufficient to produce responses in the coronary arteries without systemic effects.[6 10 18–20] The present data reveal that pretreatment of nipradilol reduces large coronary artery constriction induced by acetylcholine. It has been reported that nipradilol possesses a glyceryl trinitrate like vasodilating action and weak α adrenoceptor blocking action, in addition to a non-selective β adrenoceptor blocking action.[6] It is known that β adrenoceptor agonists relax porcine coronary arteries and β adrenoceptor antagonists constrict them in vitro.[4 21] At present, the effects of α adrenoceptor agonists and antagonists on the coronary artery are controversial.[22–24] The present study revealed that bunazosin, as an α1 adrenoceptor antagonist,[15] did not prevent porcine coronary artery constriction induced by acetylcholine. These data are consistent with the view that methoxamine (α1 adrenoceptor agonist) has no effect on relaxing porcine coronary artery in vitro.[25] It is well known that isosorbide dinitrate, a long acting nitrate, prevents the constriction of coronary artery in humans.[18 19] In the present in vivo study, both isosorbide dinitrate and nipradilol prevented the constriction of porcine large coronary artery induced by acetylcholine. This suggests that the preventive effects of nipradilol on large coronary artery constriction in swine are not due to α adrenoceptor or β adrenoceptor blocking actions, but to nitrate like vasodilating actions. However, the protective effects of nipradilol and isosorbide dinitrate were different; isosorbide dinitrate prevented the constriction of large and small coronary arteries induced by acetylcholine, but nipradilol prevented only the constriction of the large coronary artery. Nipradilol has not only a nitrate like vasodilating action but also an effect as a non-selective β adrenoceptor antagonist.[6] β Adrenoceptor antagonists constrict porcine large and small coronary arteries,[21] but the nitrate like vasodilating action appears more strongly in the large than in the small coronary arteries.[6] As a result, nipradilol may not have protective effects on small coronary artery constriction.

CLINICAL IMPLICATIONS

The administration of adrenoceptor antagonists is known to be effective in cases of effort angina, because they reduce cardiac contractility and cardiac work.[1 2 4] In addition, they are known to be the ranges of first choice in the treatment of hypertension.[3] However, treatment with β adrenoceptor antagonists may be a difficult problem if the patients have coronary artery spasm, because these agents are frequently detrimental in vasospastic angina.[4 5] The results of the present study suggest that nipradilol prevents constriction of epicardial coronary artery by its nitrate action despite its β adrenoceptor blocking action and that the agent has no significant protective effects on small coronary artery constriction. Thus, these pharmacological properties of nipradilol suggest that this agent might be more useful in the treatments of angina pectoris and hypertension with or without coronary artery spasm than other β adrenoceptor antagonists.

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Table I Haemodynamic changes. Data are means (SD)

	(a) Nipradilol group		
	Control	*ACh*	*Nip+ACh*
sAoP(mm Hg)	114(12)	116(19)	114(15)
dAoP(mm Hg)	75(10)	74(16)	76(12)
HR (beats·min^{-1})	104(11)	106(13)	104(15)
	(b) ISDN group		
	Control	*ACh*	*ISDN+ACh*
sAoP(mm Hg)	117(10)	114(11)	115(7)
sAoP(mm Hg)	78(9)	73(12)	71(6)
HR (beats·min^{-1})	108(13)	107(10)	108(11)
	(c) Bunazosin group		
	Control	*ACh*	*Bun+ACh*
sAoP(mm Hg)	114(11)	108(13)	109(9)
dAoP(mm Hg)	82(10)	78(11)	75(10)
HR (beats·min^{-1})	112(13)	103(10)	115(12)

ACh=maximum dose of acetylcholine (100 or 200 μg); Nip+ACh=ACh with nipradilol (10 μg); ISDN+ACh=ACh with isosorbide dinitrate (2.5 mg); Bun+ACh=ACh with bunazosin (100 μg); all drugs given intracardially. sAoP=systolic aortic pressure; dAoP=diastolic aortic pressure; HR=heart rate. No significant differences were found between control, ACh, or ACh with nipradilol, ISDN or bunazosin in any of the groups.

Table II Effect of nipradilol (Nip) on large and small coronary artery constriction induced by intracoronary injection of acetylcholine (ACh)

	% Coronary artery narrowing (Control=0%)+grade of blood flow delay[a]						
		ACh					
Pig No	*Control* %/-(s)	*12.5 μg* %/-(s)	*25 μg* %/-(s)	*50 μg* %/-(s)	*100 μg* %/-(s)	*200 μg* %/-(s)	*ACh with Nip (10 μg ic)* %/-(s)
1	0/N(1.2)	18/M(7.3)	29/M(12.9)	53/M(19.1)	67/M(24.2)	79/M(36.3)	36/M(33.7)[b]
2	0/N(0.9)	14/S(3.2)	28/M(8.8)	46/M(16.2)	65/M(20.8)	78/M(23.7)	30/M(25.5)[b]
3	0/N(1.7)	8/S(2.5)	23/M(7.4)	39/M(9.6)	55/M(14.4)	66/M(17.9)	37/M(15.4)[b]
4	0/N(1.8)	4/S(2.4)	19/S(5.5)	26/M(9.8)	44/M(18.6)	57/M(25.7)	32/M(22.8)[b]
5	0/N(0.9)	7/S(2.1)	20/S(6.5)	25/M(10.2)	36/M(11.5)	44/M(14.8)	19/M(15.1)[b]

% Coronary narrowing was calculated from the measured luminal diameter of the major coronary artery as the control 0%.
[a]Time required for contrast medium to reach the posterior descending coronary artery from the ostium of the right coronary artery
[b]200 μg ACh injected.
Grade of blood flow delay was as follows: N=within normal limits (<1.8 s); S=slight blood flow delay (1.8-7.0 s); M=marked blood flow delay (>7.0 s); ic=intracardiac.

Table III Effect of isosorbide dinitrate (ISDN) on large and small coronary artery constriction induced by intracoronary injection of acetylcholine (Ach)

	% Coronary artery narrowing (Control=0%)+the grade of blood flow delay[a]						
		Ach					
Pig No	*Control* %/-(s)	*12.5 μg* %/-(s)	*25 μg* %/-(s)	*50 μg* %/-(s)	*100 μg* %/-(s)	*200 μg* %/-(s)	*ACh with ISDN (2.5 mg ic)* %/-(s)
1	0/N(1.7)	14/S(4.0)	29/M(8.2)	58/M(17.8)	80/M39.7)	—	23/M(10.4)[b]
2	0/N(1.8)	9/S(2.5)	29/M(10.3)	55/M(21.0)	71/M(26.7)	84/M(41.6)	27/M(22.7)[c]
3	0/N(1.3)	5/S(1.9)	21/S(4.6)	34/M(13.7)	56/M(27.8)	68/M(28.2)	19/M(21.4)[c]
4	0/N(1.4)	18/S(4.1)	33/M(12.0)	40/M(16.2)	47/M(18.0)	58/M(25.9)	12/M(18.2)[c]
5	0/N(0.7)	5/S(2.6)	17/S(3.5)	24/S(5.0)	28/M(7.1)	32/M(7.6)	10/S(2.7)[c]

% Coronary narrowing was calculated from the measured luminal diameter of the major coronary artery as the control 0%.
[a]Time required for contrast medium to reach the posterior descending coronary artery from the ostium of the right coronary artery.
[b]100 μg ACh injected.
[c]200 μg ACh injected.
Grade of blood flow delay was as follows: N=within normal limits (<1.8 s); S=slight blood flow delay (1.8-7.0 s); M=marked blood flow delay (>7.0 s); ic=intracardiac.

Table IV *Effect of bunazosin (Bun) on large and small coronary artery constriction induced by intracoronary injection of acetylcholine (ACh)*

	% Coronary artery narrowing (Control=0%)+the grade of blood flow delay[a]						
		ACh					ACh with
Pig No	Control %/-(s)	12.5 μg %/-(s)	25 μg %/-(s)	50 μg %/-(s)	100 μg %/-(s)	200 μg %/-(s)	Bun (100 μg ic) %/-(s)
1	0/N(1.8)	7/S(2.9)	27/S(6.9)	40/M(14.5)	61/M(20.0)	82/M(29.4)	78/M(28.0)[b]
2	0/N(1.6)	14/S(3.9)	26/M(7.1)	57/M(19.8)	65/M(24.3)	78/M(27.4)	77/M(26.6)[b]
3	0/N(1.6)	11/S(3.0)	20/M(7.7)	44/M(20.8)	58/M(24.1)	62/M(26.3)	65/M(27.7)[b]
4	0/N(1.2)	19/M(7.3)	28/M(11.3)	39/M(21.1)	55/M(25.6)	58/M(31.4)	60/M(30.1)[b]
5	0/N(0.7)	4/S(1.9)	14/S(2.7)	24/S(5.7)	33/M(10.1)	46/M(11.4)	44/M(10.6)[b]

% Coronary narrowing was calculated from the measured luminal diameter of the major coronary artery as the control 0%.
[a]Time required for contrast medium to reach the posterior descending coronary artery from the ostium of the right coronary artery.
[b]200 μg ACh injected.
Grade of blood flow delay was as follows: N=within normal limits (≤1.8 s); S=slight blood flow delay (1.8-7.0 s); M=marked blood flow delay (>7.0 s); ic=intra cardiac.

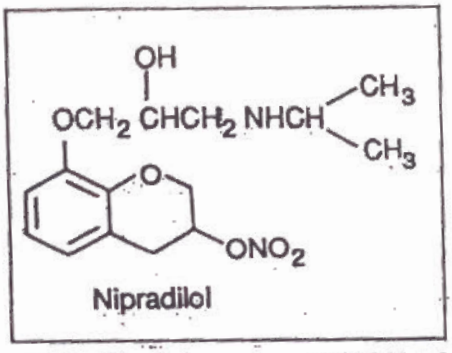

Figure 1 Chemical structure of nipradilol. Note that it possesses a nitroxy group and an oxypropranolamine side chain.

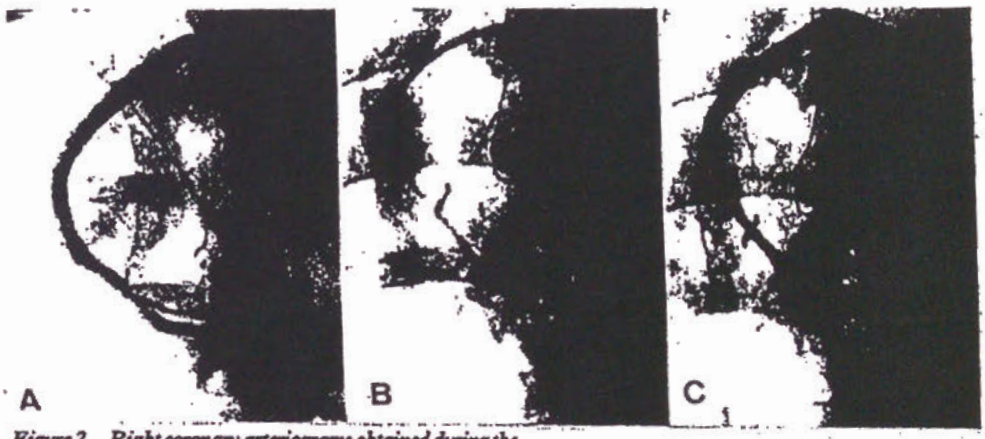

Figure 2 Right coronary arteriograms obtained during the control state (A), after intracoronary injection of 200 μg of acetylcholine (ACh) without nipradilol (B), and after pretreatment with intracardiac nipradilol (10 μg) (C). Note that 50-75% diffuse narrowing was observed after ACh infusion without nipradilol, and that by pretreatment with nipradilol the diffuse narrowing was prevented to 25-50%.

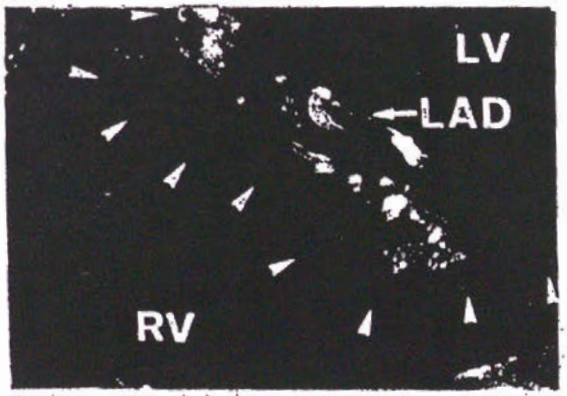

Figure 3 Photograph of clear demarcation (arrows) of the right ventricular wall supplied by the right coronary artery from the area supplied by the left coronary artery. Note that the former was anaemic (ischaemic). LAD=left anterior descending coronary artery, LV=left ventricular wall, RV=right ventricular wall.

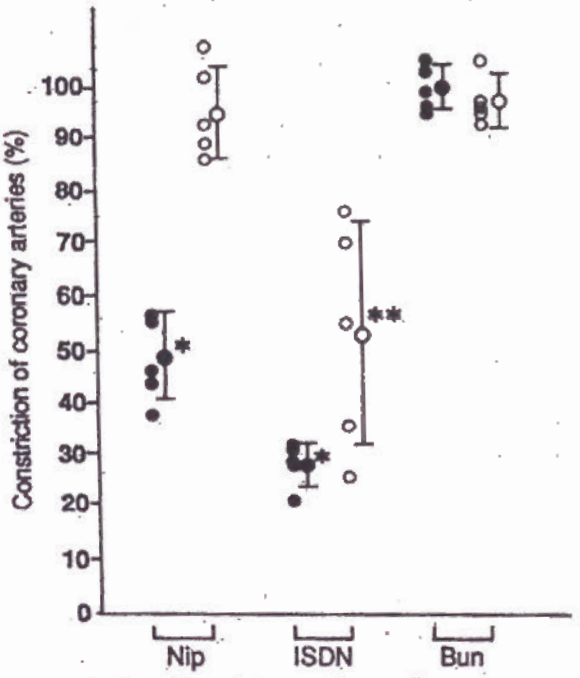

Figure 4 Percent constriction of large and small coronary arteries induced by acetylcholine (ACh) with pretreatment of nipradilol, isosorbide dinitrate (ISDN), and bunazosin. % Constriction of large coronary artery (●) is indicated by the ratio of the % narrowing of the epicardial major coronary artery pretreated with nipradilol, ISDN or bunazosin to that without pretreatment (control ACh infusion = 100%); % constriction of small coronary artery (○) is indicated by the ratio of time delay in the coronary blood flow with pretreatment of the agents to that without pretreatment (control ACh infusion = 100%). Note the significant ($p<0.01$) constrictions of large coronary arteries with nipradilol [49(8)%] and ISDN [28(4)%]. The % constriction of small coronary arteries was not significant with nipradilol, but was with ISDN. The % constriction of large and small coronary arteries with bunazosin were not significant. Nip = nipradilol group, ISDN = ISDN group, Bun = bunazosin group.
**$p<0.05$; †$p<0.001$*